(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
18 September 2003 (18.09.2003)

PCT

(10) International Publication Number
WO 03/075931 A1

(51) International Patent Classification[7]: A61K 31/575, 31/66, A61P 5/48

(21) International Application Number: PCT/CA03/00369

(22) International Filing Date: 14 March 2003 (14.03.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/098,745 14 March 2002 (14.03.2002) US

(71) Applicant: **FORBES MEDI-TECH INC.** [CA/CA]; 200-750 West Pender Street, Vancouver, British Columbia V6C 2T8 (CA).

(72) Inventors: **PRITCHARD, Haydn, P.**; 3286 West 38th Avenue, Vancouver, British Columbia V6N 2X6 (CA). **WASAN, Kishor, M.**; #607-518 West 14th Avenue, Vancouver, British Columbia V4Z 5N2 (CA). **LUKIC, Tatjana**; 1008-1160 Haro Street, Vancouver, British Columbia V6E 1E2 (CA).

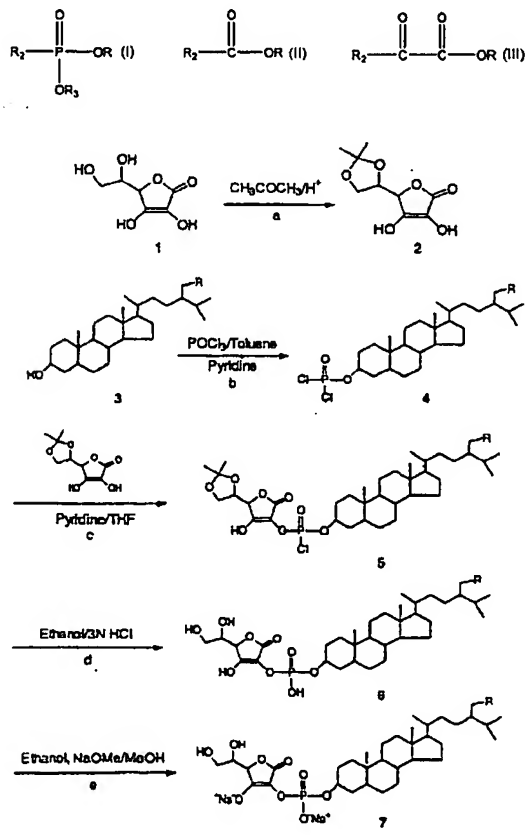
(74) Agent: **BEN-OLIEL, Susan, M., M.**; 2983 West 41st Avenue, Vancouver, British Columbia V6N 3C8 (CA).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO,

*[Continued on next page]*

(54) Title: A METHOD OF TREATING DIABETES MELLITUS INCLUDING CONDITIONS ASSOCIATED WITH DIABETES MELLITUS AND COMPLICATIONS OF DIABETES MELLITUS




(57) Abstract: A method for the treatment of diabetes mellitus and conditions associated with diabetes mellitus in an animal comprises administering a non-toxic and therapeutically effective amount of one or more of the following compounds: formula (I, II, III). wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

WO 03/075931 A1

SE, SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

**Published:**

— *with international search report*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

*For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.*

# A METHOD OF TREATING DIABETES MELLITUS INCLUDING CONDITIONS ASSOCIATED WITH DIABETES MELLITUS AND COMPLICATIONS OF DIABETES MELLITUS

## FIELD OF THE INVENTION

This present invention relates to methods of treating diabetes mellitus in animals, particularly humans.

## BACKGROUND OF THE INVENTION

Diabetes mellitus is a heterogeneous primary disorder of carbohydrate metabolism with multiple etiologic factors that generally involve insulin deficiency or insulin resistance or both. Type I, or juvenile onset, or insulin-dependent diabetes mellitus, is present in patients with little or no endogenous insulin secretory capacity. These patients develop extreme hyperglycemia (glucose accumulation in the bloodstream) and are entirely dependent on exogenous insulin therapy for immediate survival. Type 2 also called adult onset, or non-insulin-dependent diabetes mellitus ("NIDDM"), occurs in patients who retain some endogenous insulin secretory capacity, however the great majority of them are both insulin deficient and insulin resistant. Insulin resistance can be due to insufficient insulin receptor expression, reduced insulin-binding affinity, or any abnormality at any step along the insulin signaling pathway (1)

Overall, in the United States the prevalence of diabetes is probably between 2 and 4 per cent, with Type I comprising 7 to 10 per cent of all cases. Secondary complications of diabetes have serious clinical implications. Approximately 25 per cent of all new cases of end-stage renal failure occur in patients with diabetes. About 20,000 amputations (primarily of toes, feet, and legs) are carried out in patients with diabetes, representing approximately half of the non-traumatic

amputations performed in the United States. Furthermore, diabetes is the leading cause of new cases of blindness, with approximately 5000 new cases occurring each year.

Carbohydrates from food are broken down in the intestine to glucose and other simple sugars. These sugars are absorbed into the bloodstream and carried (along with other nutrients) to all the cells of the body. But in order to take up glucose from the blood, the cells need insulin -- a hormone made in the pancreas. The pancreas, a large gland located behind the stomach, has multiple functions. It produces digestive enzymes -- proteins that help break down food in the intestine. It also contains specialized groups of cells called "islets of Langerhans". These islet cells are of several types, each producing a different hormone. There are two main hormones that regulate blood glucose levels -- insulin and glucagon. Both are produced in the islets of Langerhans -- glucagon by alpha cells, and insulin by beta cells.

As glucose pours into the bloodstream after a meal, the rising blood glucose level signals the beta cells to secrete insulin. Insulin (along with the glucose) is carried by the bloodstream to cells throughout the body. Various body tissues -- especially muscle, liver, and fat -- have specialized molecules called insulin receptors on their cell surfaces. Insulin binds to these receptors, like a key in a lock -- opening channels that allow glucose to enter the cells.

Once glucose is inside the cells, it can be used for energy and growth. Excess glucose is stored in the liver in the form of a complex carbohydrate called glycogen. Meanwhile, as blood glucose levels fall, insulin secretion slows down. When the blood glucose level starts to get low, it signals the alpha cells to secrete glucagon. Glucagon, in turn, signals the liver to convert glycogen back into glucose -- a process called glycogenolysis. This prevents the blood glucose level from dropping too low to ensure that the body's cells have a steady supply of glucose between meals. Glucagon also stimulates the liver to make new glucose out of other nutrients, such as amino acids (protein building blocks) by a process called

gluconeogenesis thereby ensuring a backup source of glucose until the next meal.

The interaction of glucose, insulin, and glucagon normally ensures that blood glucose levels stay within certain limits. With diabetic individuals, however, this delicate balance is upset .

To date, insulin is the primary mode of therapy in all patients with Type I and in many with Type 2 diabetes. Depending on the number of injections per day and type(s) of insulin used, the regimen can be more or less intensive. The most intensive method consists of constant insulin delivery into a subcutaneous site in the abdominal wall via an open loop delivery device consisting of a small insulin pump that must be worn by the patient essentially 24 hours a day. Oral hypoglycemic agents such as sulfonylureas are effective in Type II patients but approximately 10 to 20 percent of patients do not respond or cease to respond 12-24 months after beginning treatment.

Effective control of glucose level is difficult to achieve for prolonged periods even with the most meticulous mode of insulin therapy in the most motivated patients. Transplantation of the pancreas or islet cells, which normally produce insulin, continues to receive extensive study as a potential treatment. In addition, efforts towards developing newer and better external or implantable insulin-delivery devices integrated with a glucose sensor continues.

Type 2 diabetes usually begins in adulthood (typically after age 40), but also can occur in younger people. Generally, in people having this condition, the pancreas produces insulin but the body's cells are unable to respond to it effectively. This is called insulin resistance. A person can have insulin resistance without diabetes, as long as there is enough insulin to overcome the resistance. But if insulin resistance continues to increase and/or insulin production falls below the amount needed to compensate, diabetes will develop.

Most type 2 diabetics can be treated with oral medications that boost pancreatic

insulin secretion and/or lower insulin resistance. But over time, the pancreatic beta cells may become less and less responsive thereby secreting less insulin when blood glucose levels are high. Eventually, the person may need insulin injections to help control blood glucose levels.

Because persistent hyperglycemia can interfere with tissue healing and immunity, in some people the first sign of diabetes mellitus may be slow-healing sores, frequent infections, or gum disease. Or the first symptoms to be noticed may be those of organ damage, such as heart disease or neuropathy. Common symptoms of diabetic neuropathy include tingling, loss of sensation, or burning pain in the feet and legs. Although the exact causes are not known, diabetes mellitus appears to result from an interaction between genes and lifestyle factors.

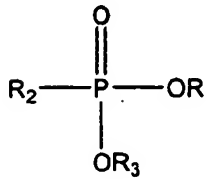
One of the most important risk factors for diabetes mellitus is being overweight.(2). Research has shown that being overweight and having a high fat diet is associated with insulin resistance (3). This is especially true when the excess fatty tissue is located in the upper part of the body, around the abdomen -- an "apple shape" (as opposed to a "pear shape" with excess fat below the waist). While the reasons for this are not yet well understood, researchers believe that insulin resistance is increased by hormones (such as leptin and resistin) that are secreted by fat cells.

Within the field, there is continued and on-going research to develop safe and effective treatments for diabetes. It is an object of the present invention to obviate or mitigate the disadvantages and insufficiencies known in the field.

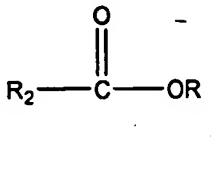
## SUMMARY OF THE INVENTION

The present invention provides a method for the treatment of diabetes mellitus and

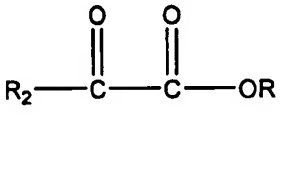
conditions associated with diabetes mellitus in an animal, which method comprises administering a non-toxic and therapeutically effective amount of one or more of the following compounds:



I



II



III

wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

The present invention also provides for the use of these compounds in regulating serum glucose levels, in enhancing cellular insulin sensitivity and in enhancing glucose responsiveness and level of insulin secretions of pancreatic beta cells.

The compounds of the present invention have been found to be surprisingly effective in improving glucose tolerance in animals. Until now, there has been no appreciation of these types of compounds having such effects.

One significant advantage of the use of the particular compounds of the present invention for this purpose is their water-solubility. In particular, it has been found that solubility in aqueous solutions such as water is excellent, thereby allowing oral administration *per se* without any further enhancements or modifications. Accordingly, the compounds of the present invention can be prepared and used as such or they can be easily incorporated into foods, beverages, pharmaceuticals and nutraceuticals regardless of whether these "vehicles" are water-based. This enhanced solubility

generally translates into lower administration dosages of the compounds in order to achieve the desired therapeutic or prophylactic effect.

These effects and other significant advantages are described in more detail below.

**BRIEF DESCRIPTION OF THE DRAWINGS**

The present invention is illustrated by way the following non-limiting drawings in which:

Figure 1 is a schematic showing a process of preparing phytostanol-phosphate-ascorbate and its sodium salt;

Figure 2 is a schematic showing a process of preparing phytostanol-carbonate-ascorbate and its sodium salt;

Figure 3 is a schematic showing a process of preparing phytostanol-oxalate-ascorbate and its sodium salt;

Figure 4 is a graph showing the effect of sitostanol-phosphoryl-ascorbate ("FM-VP4") treatment on body weight and morning blood glucose levels within lean and fatty Zucker rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. fatty;

Figure 5 is a is a graph showing the effect of sitostanol-phosphoryl-ascorbate ("FM-VP4") treatment on morning blood glucose levels within lean and fatty Zucker rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. fatty;

Figure 6 is a graph showing the effect of FM-VP4 following 30-day treatment (after) on plasma leptin levels within lean and fatty Zucker rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. Before;

Figure 7 is a graph showing the effect of FM-VP4 following 30-day treatment on glucose tolerance in lean Zucker rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. Lean control;

Figure 8 is a graph showing the effect of FM-VP4 30-day treatment on glucose tolerance in fatty Zucker (VDF) rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. VDF control;

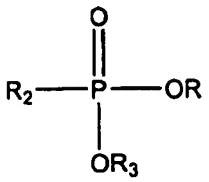
Figure 9 is a graph showing the effect of FM-VP4 following 30-day treatment on OGTT insulin in lean Zucker rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. Lean control; and

Figure 10 is a graph showing the effect of FM-VP4 following 30-day treatment on OGTT insulin in fatty Zucker rats. Data presented as mean ± standard deviation, n=6 in each treatment group. *p<0.05 vs. fatty

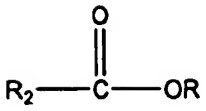
**PREFERRED EMBODIMENTS OF THE INVENTION**

The following detailed description is provided to aid those skilled in the art in practising the present invention. However, this detailed description should not be construed so as to unduly limit the scope of the present invention. Modifications and variations to the embodiments discussed herein may be made by those with ordinary skill in the art without departing from the spirit or scope of the present invention.

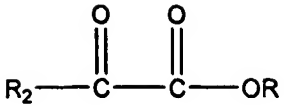
According to the present invention, there is provided a method for the treatment of diabetes mellitus and conditions associated with diabetes mellitus in an animal, which method comprises administering a non-toxic and therapeutically effective amount of one or more the following compounds:



I



II



III

wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

The term "therapeutically effective" is intended to qualify the amount of the compound(s) administered in order to achieve one or more of the following goals:

a) treating conditions associated with diabetes such as hyperglycaemia, and insulin resistance, including acquired insulin resistance;
b) treating complications of diabetes mellitus such as insulin resistance, including hereditary insulin resistance, impaired glucose tolerance and hyperinsulinaemia;
c) treating conditions associated with insulin resistance include polycystic ovarian syndrome and steroid induced insulin resistance and gestational diabetes;
d) treating complications associated with diabetes mellitus' includes renal diseases, especially renal disease associated with Type 2 diabetes, neuropathy and retinopathy. Renal diseases associated with Type 2 diabetes include nephropathy, glomerulonephritis, glomerular sclerosis, hypertensive nephrosclerosis and end stage renal disease. Additional renal diseases associated with Type 2 diabetes include nephrotic syndrome;
e) improving glucose tolerance;
f) regulating serum glucose levels;
g) enhancing cellular insulin sensitivity;
h) enhancing glucose responsiveness and level of insulin secretions of pancreatic beta cells; and

i) treating pre-diabetic conditions.

Diabetes mellitus is preferably Type 2 diabetes.

The elements of the compounds will be described in more detail below. It should be noted that, throughout this disclosure, the terms "compound" "derivative", "structure" and "analogue" may be used interchangeably to describe the structures above which link both a phytosterol or phytostanol and ascorbic acid and which have been found to be effective in the treatment of diabetes mellitus, conditions associated with diabetes mellitus and complications associated with diabetes mellitus.

Phytosterols/Phytostanols

As used herein, the term "phytosterol" includes all phytosterols without limitation, for example: sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers. The term "phytostanol" includes all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers. It is to be understood that modifications to the phytosterols and phytostanols i.e. to include side chains also falls within the purview of this invention. It is also to be understood that, when in doubt throughout the specification, the term "phytosterol" encompasses both phytosterol and phytostanol i.e. the terms may be used interchangeably unless otherwise specified.

The phytosterols and phytostanols for use in forming derivatives in accordance with this invention may be procured from a variety of natural sources. For example, they may be obtained from the processing of plant oils (including aquatic plants) such as corn oil and other vegetable oils, wheat germ oil, soy extract, rice extract, rice bran, rapeseed oil, sunflower oil, sesame oil and fish (and other marine-source) oils. The present invention is not to be limited to any one source of phytsterols. US Patent Serial No. 4,420,427 teaches the preparation of sterols from vegetable oil sludge using solvents such as methanol. Alternatively, phytosterols and phytostanols may be obtained from tall oil pitch or soap, by-products of forestry practises as described in

US Patent Serial No.5,770,749, incorporated herein by reference.

In one preferred form, the derivative of the present invention is formed with naturally-derived or synthesized beta-sitosterol, campestanol, sitostanol and campesterol and each of these derivatives so formed may then be admixed a composition prior to delivery in various ratios. In another preferred form, the derivative of the present invention is formed with naturally-derived or synthesized sitostanol or with naturally derived or synthesized campestanol or mixtures thereof.

In a preferred form, sitostanol is the phytostanol. In a most preferred form, the compound is any phytostanol-phosphoryl ascorbate or salts thereof.

R2

R2 comprises ascorbic acid or any derivative thereof. What is achieved within the scope of the present invention is the creation of a new structure or compound wherein a phytosterol or phytostanol moiety is chemically linked to ascorbic acid. The union benefits and enhances the both parts of this new structure. The phytosterol moiety, formerly poorly soluble, becomes, as part of the new derivative, much more readily soluble in aqueous and non-aqueous media such as oils and fats. Accordingly, administration of the phytosterol becomes possible without any further enhancements to modify its delivery.

R3

R3 may be hydrogen or may convert the parent compound into a salt . The over-riding consideration in the selection of the appropriate salt is that they are acceptable pharmaceutically, nutraceutically or for use in foods, beverages and the like. Such salts must have an acceptable anion or cation. Within the scope of the present invention, suitable acid addition salts include those derived from inorganic acids such as hydrochloric, hydrobromic, phosphoric, metaphosphorice, nitric, sulfonic and sulfuric acids and organic acids such as acetic, benzenesulfonic, benzoic, citric, ethanesulfonic, fumaric, gluconic, glyconic, glycolic, isothionic, lactic, lactobionic, maleic, malic, methanesulfonic, succinic, toluenesulfonic, and tartaric.

Suitable base salts include ammonium salts, or any salt of a metal, alkali earth metal or alkali metal. Preferably, R3 is selected from one of: calcium, magnesium, manganese, copper, zinc, sodium, potassium and lithium. Most preferably, R3 is sodium.

In a most preferred form of the present invention, the compound is structure 1 noted above, the phytostanol is sitostanol and R3 is sodium.

**Derivative Formation**

**a) Ester Formation**

There are many processes by which structures comprising phytosterols and/or phytostanols and ascorbic acid can be formed. In general, the selected phytosterol or stanol (or halophosphate, halocarbonate or halo-oxalate derivatives thereof) and ascorbic acid are mixed together under reaction conditions to permit condensation of the "acid" moiety with the "alcohol" (phytosterol). These conditions are the same as those used in other common esterification reactions such as the Fisher esterification process in which the acid component and the alcohol component are allowed to react directly or in the presence of a suitable acid catalyst such as mineral acid, sulfuric acid, phosphoric acid, p-toluenesulfonic acid. The organic solvents generally employed in such esterification reactions are ethers such as diethyl ether, tetrahydrofuran, or benzene, toluene or similar aromatic solvents and the temperatures can vary from room to elevated temperatures depending on the reactivity of the reactants undergoing the reaction.

In a preferred embodiment, the process to form the ester derivative comprises "protecting" the hydroxyl groups of the ascorbic acid or derivatives thereof as esters (for example, as acetate esters) or ethers (for example, methyl ethers) and then condensing the protected ascorbic acid with the phytosterol/phytostanol halophospahte, halocarbonate or halo-oxalate under suitable reaction conditions. In

general, such condensation reactions are conducted in an organic solvent such as diethyl ether, tetrahydrofuran, or benzene, toluene or similar aromatic solvents. Depending on the nature and reactivity of the reactants, the reaction temperatures may vary from low (-15°C) to elevated temperatures.

Figure 1 is a schematic showing the formation of the "protected" ascorbic acid (step a), the formation of the intermediary chlorophosphate/stanol derivative (step b), and the condensation reaction (alternatively steps c or d) yielding one of novel derivatives of the present invention based on formula I: phytostanol-phosphate-ascorbate (noted as structure 6).

In more detail, the process shown in Figure 1 is as follows: ascorbic acid is initially protected from decomposition by the formation of 5,6-isopropylidene-ascorbic acid (structure 2). This can be achieved by mixing acetone with ascorbic acid and an acidic catalyst such as sulfuric acid or hydrochloric acid under suitable reaction conditions (refer to Example 1 below). Phytostanol chlorophosphate (structure 4) is prepared by forming a solution of phytostanol in toluene and pyridine (although other nitrogen bases such as aliphatic and aromatic amines may alternatively be used) and treating this solution with a phosphorus derivative such as phosphorus oxychloride. The residue so formed after filtration and concentration of the mother liquor is phytostanol chlorophosphate (structure 4). The latter is then mixed with 5,6-isopropylidene-ascorbic acid and, after the addition of a suitable alcohol such as ethanol and HCl (step d), concentrated. Alternatively, pyridine/THF may be added (step c) and the product concentrated. After final washing and drying (step e), the resultant novel product of both steps c or d is phytostanol-phosphate-ascorbate (structure 6).

Alternatively, ascorbic acid is protected at the hydroxyl sites not as 5,6-isopropylidene-ascorbic acid but as esters (for example as acetates, phosphates and the like..). The latter may then be condensed with phytosterols or phytostanols, derivatized as described above, using known esterification methods ultimately to produce the structures of the present invention. The formation of mono and diphosphates of ascorbic acid is described thoroughly in the literature. For example, US Patent Serial

No. 4,939,128 to Kato et al., the contents of which are incorporated herein by reference, teaches the formation of phosphoric acid esters of ascorbic acid. Similarly, US Patent Serial No. 4,999,437 to Dobler et al., the contents of which are also fully incorporated herein by reference, describes the preparation of ascorbic acid 2-phosphate. In Dobler et al., the core reaction of phosphorylating ascorbic acid or ascorbic acid derivatives with POCl3 in the presence of tertiary amines (described in German Laid Open Application DOS 2,719,303) is improved by adding to the reaction solution a magnesium compound, preferably an aqueous solution of a magnesium compound. Any of these known ascorbic acid derivatives can be used within the scope of the present invention.

Figure 2 is a schematic showing the formation of the "protected" ascorbic acid (step a), the formation of the intermediary chlorocarbonate/stanol derivative (step b), and the condensation reaction (optionally steps c or d) yielding structure 9 (10 is the same), one of derivatives of the present invention based on formula II: phytostanol-carbonate-ascorbate. These chlorocarbonate derivatives may be prepared by the same process outlined in detail above with respect to Figure 1; however, the phosphorus oxylchloride is replaced (as shown in step b of Figure 2) by phosgene.

Figure 3 is a schematic showing the formation of the "protected" ascorbic acid (step a), the formation of the intermediary chloro-oxalate/stanol derivative (step b), and the condensation reaction (optionally steps c or d) yielding a novel structure 13 (same as 14), one of the derivatives of the present invention based on formula III: phytostanol-oxalate-ascorbate (noted as structure 14). These chloro-oxalate derivatives may be prepared by the same process outlined in detail above with respect to Figure 1; however, the phosphorus oxylchloride is replaced (as shown in step b of Figure 3) by oxalyl chloride.

**b) Salt Formation**

The present invention encompasses not only the parent structures comprising phytosterols or phytostanols and ascorbic acid (for example, those preferred structures shown as structures 5 and 6 in Figure 1, structures 9 an 10 in Figure 2 and structures

13 and 14 in Figure 3) but also the salts thereof. These salts are even more water soluble than the corresponding parent compounds and therefore their efficacy and evaluation both *in vitro* and *in vivo* is much improved.

Salt formation of the derivatives of the present invention can be readily performed by treatment of the parent compound with a series of bases (for example, sodium methoxide or other metal alkoxides) to produce the corresponding alkali metal salts. Other metal salts of calcium, magnesium, manganese, copper, zinc, and the like can be generated by reacting the parent with suitable metal alkoxides. With respect to formula I, R3 represents either hydrogen (parent compound) or any metal, alkali earth metal, or alkali metal (the salt).

**c) Reduction by Catalytic (Hydrogenation) and Chemical Methods**

Optionally, the phytosterol derivatives of the present invention or the constituent moieties thereof (either the phytosterol or the ascorbic acid) prior to or after derivative formation may be hydrogenated or saturated. The hydrogenation of heterocyclic ring systems to the partially or fully reduced analogues is a well known process. For example, the catalytic and/or chemical reduction of the ring of ascorbic acid to the corresponding dihydro analogue is readily accomplished under an atmosphere of hydrogen and a metal catalyst such as platinum, palladium or Raney Nickel. In general, this reduction is performed in an organic solvent such as ethanol, ethyl acetate or similar media and either under atmospheric pressure or at a low pressure (3-5 psi) at room temperature or slightly elevated temperatures.

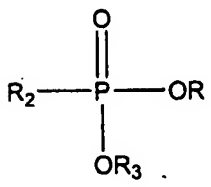
The chemical reductions of such systems involve reduction with a family of "hydride" reagents such as sodium borohydride, lithium aluminum hydride and their analogues. These reductions are generally performed in an anhydrous inert medium involving ethyl ether, tetrahydrofuran, dioxane, or benzene, toluene or similar aromatic solvents at room to reflux temperatures.

Similar catalytic or chemical processes can be applied to all of the phytosterol analogues of the present invention. Accordingly, the compounds for use within the

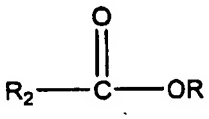
methods of the present invention include all fully or partially reduced derivatives wherein the ring of ascorbic acid is partially or fully reduced and/or wherein the phytosterol moiety is fully or partially hydrogenated.

**Derivatives**

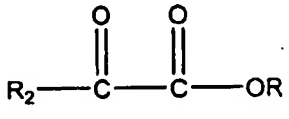
The present invention comprises a method of treatment of diabetes mellitus and conditions associated with diabetes mellitus in an animal, a method of regulating serum glucose levels and enhancing cellular insulin sensitivity and a method of enhancing glucose responsiveness and level of insulin secretions of pancreatic beta cells which method comprise administering one or more of the derivatives comprising phytosterol and/or phytostanol and ascorbic acid, including salts thereof represented by the general formulae:



I



II



III

wherein R is a phytosterol or phytostanol moiety; R2 is derived from ascorbic acid and R3
is hydrogen or any metal, alkali earth metal, or alkali metal. Most preferably, compounds are the halophosphate, halocarbonate and halo-oxalate/phytostanol/ascorbate derivatives as shown in Figures 1 through 3 as structures 5, 6, 7, 9, 10, 11 13 14 and 15. It is to be clearly understood; however, that these structures are only a selection of the many novel derivatives which fall within the purview of formulae I, II and III and which may be used in accordance with the methods of the present invention. It is also to be understood that although sodium salts are shown in structures 7, 11 and 15, other salts are included within the

scope of the invention, as described above.

**Mechanism of Action**

While not intending to be bound by any one theory as to the various therapeutic efficacies of the compounds described herein, it appears that much rests on the improved glucose tolerance afforded by these compounds.

Data provided in the examples below show that there is a highly significant improvement in glucose tolerance in the fat group that exhibit a diabetic glucose tolerance curve. Although insulin response to oral glucose shows no significant change in non-diabetic lean animals, there is a change in the insulin secretory profile in the fat group after treatment with the compounds of the present invention. The fat animals are very insulin resistant and are hyperinsulinemic. It is thought that the hyperinulinemia is an attempt to compensate for the lack of effectiveness of insulin on its' target tissues (insulin resistance). These animals are also characterized by a blunted insulin response to glucose. This is shown by the almost flat insulin response to oral glucose seen in Fig 5 (before study group). It is clear that the compounds of the present invention improve glucose tolerance in the fatty (genetically) diabetic rats.

**Methods of Use**

The desired effects described herein may be achieved in a number of different ways. These compounds may be administered by any conventional means available for use in conjunction with pharmaceuticals, nutraceuticals, foods, beverages, and the like.

The amount of the compound which is required to achieve the desired effects will, of course, depend on a number of factors such as the particular compound chosen, the mode of administration and the condition of the patient.

The compounds of the present invention can be administered to a patient either by themselves, or in pharmaceutical compositions where they are mixed with suitable carriers or excipients.

Use of pharmaceutically acceptable carriers to formulate the compounds herein disclosed for the practice of the invention into dosages suitable for systemic administration is within the scope of the invention. With proper choice of carrier and suitable manufacturing practice, the compounds of the present invention, in particular, those formulated as solutions, may be administered parenterally, such as by intravenous injection. The compounds can be formulated readily using pharmaceutically acceptable carriers well known in the art into dosages suitable for oral administration. Such carriers enable the compounds of the invention to be formulated as tablets, pills, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated.

Pharmaceutical compositions, comprising one or more of the compounds of the present invention, include compositions wherein the active ingredients are contained in an effective amount to achieve their intended purpose. Determination of the effective amounts is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

In addition to the active ingredients these pharmaceutical compositions may contain suitable pharmaceutically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. The preparations formulated for oral administration may be in the form of tablets, dragees, capsules, or solutions.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical formulations for parenteral administration include aqueous solutions of the active compounds in water-soluble form. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions.

Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate or triglycerides, or liposomes. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the compounds to allow for the preparation of highly concentrated solutions.

Pharmaceutical preparations for oral use can be obtained by combining the active compounds with solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used, which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty

oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added.

Oral liquid preparations may be in the form of, for example, emulsions, syrups, or elixirs, or may be presented as a dry product for reconstitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, for example sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminium stearate gel, hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate, or acacia; non-aqueous vehicles (which may include edible oils), for example almond oil, fractionated coconut oil, oily esters such as esters of glycerine, propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and if desired conventional flavouring or colouring agents.

In another form of the present invention, the compounds of the present invention may be administered through foods, beverages and nutraceuticals, including, without limitation, the following:

1) Dairy Products --such as cheeses, butter, milk and other dairy beverages, spreads and dairy mixes, ice cream and yoghurt;

2) Fat-Based Products--such as margarines, spreads, mayonnaise, shortenings, cooking and frying oils and dressings;

3) Cereal-Based Products--comprising grains (for example, bread and pastas) whether these goods are cooked, baked or otherwise processed;

4) Confectioneries--such as chocolate, candies, chewing gum, desserts, non-dairy toppings (for example Cool Whip™), sorbets, icings and other fillings;

5) Beverages-- whether alcoholic or non-alcoholic and including colas and other soft drinks, juice drinks, dietary supplement and meal replacement drinks such as those sold under the trade-marks Boost™ and Ensure™; and

6) Miscellaneous Products--including eggs and egg products, processed foods such as soups, pre-prepared pasta sauces, pre-formed meals and the like.

The compounds of the present invention may be incorporated directly and without further modification into the food, nutraceutical or beverage by techniques such as mixing, infusion, injection, blending, dispersing, emulsifying, immersion, spraying and kneading. Alternatively, the compounds may be applied directly onto a food or into a beverage by the consumer prior to ingestion. These are simple and economical modes of delivery.

## EXAMPLES

The present invention is described by the following non-limiting examples:

Example 1--Protection of ascorbic Acid

Oleum (24%, 8.3g) was added dropwise to acetone (50ml). Ascorbic acid (12g) was introduced to the mixture at 0°C and the reaction mixture was stirred at 0°C for 6 hours. The obtained crystals were filtered off under suction, the filtered cake was pressed to dryness and then washed with acetone (30ml). The product, 5,6-isopropylidene ascorbic acid (14g) was obtained.

**Example 2**-- Attachment to Phytostanols

A solution of phytostanol mixture (24g) (campestanol: 36.4%; sitostanol: 62.3%) in toluene (500ml) and pyridine (25ml) was added dropwise to a mixture of phosphorous oxychloride (9ml) in toluene (200ml) at 0°C. The mixture was stirred at room temperature for 3 hours. The pyridine hydrochloride was filtered off and the mother liquor was concentrated to recover the toluene. The residue was dissolved in dry THF (100ml) and a solution of the above prepared protected ascorbic acid (14g) in dry THF (400ml) was added dropwise at 0°C. The stirring at room temperature was maintained for 1 hr. The solution was concentrated to remove the solvent. Ethanol (400ml) and 3N HCl (200ml) were added, the mixture was heated to 50°C for 30 min and concentrated. Ethyl acetate (600ml) was added, the resultant solution was washed with water (3X300ml), dried over sodium sulfate, concentrated and the product (phytostanol-phosphate-ascorbate) was obtained as a white powder 22g.

**Example 3**--Conversion to Sodium Salt

The above prepared acid (17g) was dissolved in ethanol (100ml) and a solution of sodium methoxide (2.7g) in ethanol (50ml) was added at stirring and at room temperature. The stirring was maintained for 30 min. after the addition. The resultant white cake was filtered off, dried and weighed, to afford a white powder 20g (phytostanol-phosphate-ascorbate sodium).

**Example 4**– Studies in Zucker (fa/fa) fatty rats

Adult male Zucker (fa/fa) fatty and lean rats (340-410 g) were used in both the 4-week treatment protocol and [$^3$H]-cholesterol GI absorption studies. The rat is an appropriate animal model to investigate the gastrointestinal (GI) absorption of cholesterol following oral administration due to similarities in intestinal characteristics (i.e. anatomical, metabolic and biochemical characteristics) and intestinal processing and absorption of dietary cholesterol between rats and humans. Similar processing and absorption of dietary cholesterol is also observed in Zucker rats.

**Determination of glucose insulin, leptin and plasma lipid concentrations.**

Glucose was measured using the Surestep blood glucose monitoring system (Lifescan Canada). Insulin and leptin levels were measured by radioimmunoassay (RIA kits LINCO corp). Plasma cholesterol and triglyceride were measured using enzymatic kits (Sigma, St. Louis, MO) as previously described.

Research Design. A group of 12 age matched male obese (n=6) and lean (n=6) VDF rats were used in this study. Prior to administration of one of the compounds of the present invention, referred to as "FM-VP4" (sitostanol-phosphate-ascorbate), animals were weighed and fasted overnight and blood samples taken for the determination of: glucose, insulin, leptin, cholesterol, and triglycerides. Animals were then treated with 250 mg/kg FM-VP4 twice daily by oral gavage for 30 days (using a 2% solution of FM-VP4 dissolved in drinking water). Every two days animals were weighed and morning blood glucose levels measured. At the end of 30 days all animals were fasted overnight and a second oral glucose tolerance test performed. Fasting blood samples were taken from all animals for glucose, insulin and leptin determination. This was followed by oral glucose tolerance test (OGTT) carried out on conscious unrestrained animals as previously described. The glucose (1g/kg as a 40% solution) was given by oral gavage and blood samples taken at 10,20,30,60 and 120 minutes for glucose and insulin determination.

<u>Plasma [$^3$H]Cholesterol Concentration following FM-VP4 Administration</u>

Development of a FM-VP4-[$^3$H]cholesterol oral gavage formulation. The formulations

were composed of 25 μCi [$^3$H]-cholesterol (corresponding to 227.3 ng of cholesterol based on specific activity of 110 mCi/mg; Amersham, Mississauga, ON, Canada), 1 mg unlabeled cholesterol (6,7) and increasing amounts of FM-VP4 (1-20 mg). The formulations were mixed with 1 ml of Intralipid® (Clintec Nutritional Company; Deerfield, IL, USA) on the day of the study and gently vortexed. Intralipid® is a sterile non-pyrogenic fat emulsion prepared for administration as a source of calories and essential fatty acids, and was used as a vehicle to solubilize and co-administer exogenous [$^3$H]-cholesterol and FM-VP4 in a palpable oral formulation. Liquid chromatography-mass spectrometry analysis revealed minimal total cholesterol and vegetable stanol content within 10% Intralipid® prior to the addition of exogenous cholesterol (labeled and unlabeled) and FM-VP4 as previously published.

Differences in body weight, blood glucose, plasma leptin, glucose and insulin levels following an oral glucose tolerance test and [3H]cholesterol GI absorption between treatment and control groups were determined using an analysis of variance (PCANOVA; Human Dynamic Systems). Statistical differences were determined using the Newman Keuls post-hoc test. Differences were considered significant if $p<0.05$. All data are expressed as mean +/- standard deviation.

Results

**Body weight.** Body weight profiles over the 30-day drug treatment period (Fig. 4) indicate no significant change in body weight in either the lean or fatty group after treatment.

**Morning blood glucose.** Morning blood glucose profiles over the 30-day drug treatment period (Fig. 5) indicate no significant change in morning (fed) blood glucose in either the lean or fatty group after treatment. Variability always exists in these readings as animals may vary in feeding status relative to the time of glucose measurement (8 AM). However, in these studies blood glucose levels remained relatively consistent throughout the duration of the study. Furthermore, FM-VP4 treatment did not alter lean and fatty Zucker rat daily diet and/or water consumption (data not shown).

**Plasma leptin levels.** Levels of the satiety hormone leptin did not change

significantly after drug treatment in either group (Fig. 6). It should be noted that the primary defect in the fatty Zucker rat is a mutation in the central leptin receptors resulting in abnormal hypothalamic appetite regulation. As a consequence the fa/fa, fatty animals have greatly elevated leptin levels. There is a suggestion of reduced leptin levels after FM-VP4 treatment in fat animals but differences are not significant.

**Oral glucose tolerance test (OGTT)-Glucose data**. Figures 7 and 8 show OGTT glucose data in lean and fat animals respectively before and after FM-VP4 treatment. Whereas there is no alteration in glucose tolerance in the non-diabetic, normoglycemic lean group, there is a highly significant improvement in glucose tolerance in the fat group that exhibit a diabetic glucose tolerance curve (Fig. 8).

**Oral glucose tolerance test (OGTT)-Insulin data.** Figures 9 and 10 show OGTT insulin data in lean and fat animals respectively before and after FM-VP4 treatment. The insulin response to oral glucose shows no significant change in nondiabetic lean animals (Fig 9) whereas there is a change in the insulin secretory profile in the fat group after FM-VP4 treatment (Fig. 10).

**Discussion**

The purpose of these studies was to determine the effects of phytostanol phosphoryl ascorbate (FM-VP4) on insulin resistance and hyperglycemia in Zucker (*fa/fa*) fatty and lean rats. FM-VP4 treatment did not alter body weight, morning glucose and leptin. These findings suggest that FM-VP4 does not alter systemic and genetically predisposed glucose metabolism and hormonally regulated appetite.

In addition, whereas there is no alteration in glucose tolerance in the non-diabetic, normoglycemic lean group, there is a highly significant improvement in glucose tolerance in the fat group that exhibit a diabetic glucose tolerance curve. However, although insulin response to oral glucose shows no significant change in nondiabetic lean animals there is a change in the insulin secretory profile in the fat group after FM-VP4 treatment. The fat animals are very insulin resistant and hyperinsulinemic. It is thought that the hyperinulinemia is an attempt to compensate for the lack of

effectiveness of insulin on its' target tissues (insulin resistance). These animals are also characterized by a blunted insulin response to glucose. This is indicated by the almost flat insulin response to oral glucose seen in Fig 8 (before study group).

It is interesting that an early peak of insulin secretion at the 10-minute period following oral glucose administration accompanies improved glucose tolerance in these animals following FM-VP4 treatment. This is indicative of increased glucose responsiveness of the insulin secreting pancreatic ß cell in these animals as a result of such treatment. Loss of early phase glucose-stimulated insulin secretion is a hallmark of type 2 (adult onset diabetes) in humans and animal models of type 2 diabetes including our colony of fatty Zucker rats. Whatever the mechanism of improved glucose tolerance by FM-VP4 treatment (not due to anorexia or weight loss -see Fig. 4), the reduced hyperglycemia is likely effecting a reduction in the severity of insulin resistance and this in turn reduces the demand for insulin and results in a more responsive pancreas.

In summary, administration of FM-VP4 significant improvement glucose tolerance within fatty Zucker rats without altering body weight and morning glucose, insulin, and leptin levels in both lean and fatty rats.

**References:**

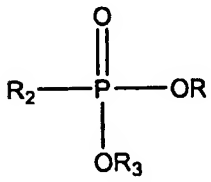
1. Olefsky, 1988, in "Cecil Textbook of Medicine," 18th Ed., 2:1360-81)

2. De Fronzo RA Ferrannini E. Insulin resistance: A multifaceted syndrome response for NIDDM, obesity, hypertension, dyslipidemia and atherolsclerotic cardiovascular disease. Diabetes Care:14:173; 1991.

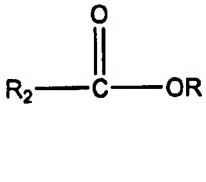
3. Beck-Nielsen, H, Pedersen, O, Sorensen NS: Effects of dietary changes on cellular insulin binding and in vivo insulin sensitivity. Metabolism 29:482: 1994.

WE CLAIM:

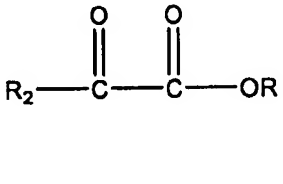
1. A method for the treatment of diabetes mellitus and conditions associated with diabetes mellitus in an animal, which method comprises administering a non-toxic and therapeutically effective amount of one or more of the following compounds:



I



II



III

wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

2. The method of claim 1 wherein the phytosterol is selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

3. The method of claim 1 wherein the phytostanol is selected from the group consisting of all saturated or hydrogenated phytosterols and all natural or synthesized

forms and derivatives thereof, including isomers.

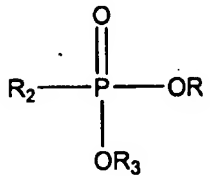
4. The method of claim 1 wherein the phytostanol is sitostanol.

5. The method of claim 1 wherein R3 is selected from the group consisting of calcium, magnesium, manganese, copper, zinc, sodium, potassium and lithium.

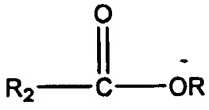
6. The method of claim 1 wherein the compound is structure 1 and the phytostanol is sitostanol.

7. The method of claim 1 wherein the animal is human.

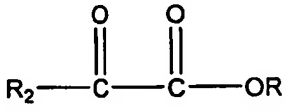
8. A method for regulating serum glucose levels in an animal, which method comprises administering a non-toxic and therapeutically effective amount of one or more of the following compounds:



I



II



III

wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

9. The method of claim 8 wherein the phytosterol is selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

10. The method of claim 8 wherein the phytostanol is selected from the group consisting of all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers.

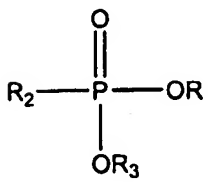
11. The method of claim 8 wherein the phytostanol is sitostanol.

12. The method of claim 8 wherein R3 is selected from the group consisting of calcium, magnesium, manganese, copper, zinc, sodium, potassium and lithium.

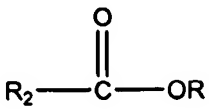
13. The method of claim 8 wherein the compound is structure 1 and the phytostanol is sitostanol.

14. The method of claim 8 wherein the animal is human.

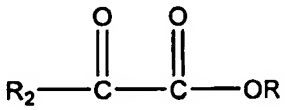
15. A method for enhancing cellular insulin sensitivity an animal, which method comprises administering a non-toxic and therapeutically effective amount of one or more of the following compounds:




I




II




III

wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

16. The method of claim 15 wherein the phytosterol is selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

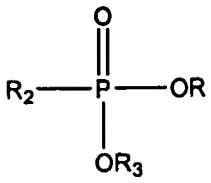
17. The method of claim 15 wherein the phytostanol is selected from the group consisting of all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers.

18. The method of claim 15 wherein the phytostanol is sitostanol.

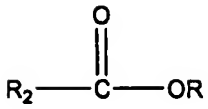
19. The method of claim 15 wherein R3 is selected from the group consisting of calcium, magnesium, manganese, copper, zinc,sodium, potassium and lithium.

20. The method of claim 15 wherein the compound is structure 1 and the phytostanol is sitostanol.

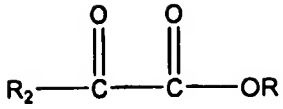
21. A method for enhancing glucose responsiveness and level of insulin secretion in animal pancreatic beta cells, which method comprises administering a non-toxic and therapeutically effective amount of one or more of the following compounds:



I



II



III

wherein R is a phytosterol or phytostanol moiety R2 is derived from ascorbic acid and R3 is hydrogen or any metal, alkali earth metal, or alkali metal; and all salts thereof.

22. The method of claim 21 wherein the phytosterol is selected from the group consisting of sitosterol, campesterol, stigmasterol, brassicasterol, desmosterol, chalinosterol, poriferasterol, clionasterol and all natural or synthesized forms and derivatives thereof, including isomers.

23. The method of claim 21 wherein the phytostanol is selected from the group consisting of all saturated or hydrogenated phytosterols and all natural or synthesized forms and derivatives thereof, including isomers.

24. The method of claim 21 wherein the phytostanol is sitostanol.

25. The method of claim 21 wherein R3 is selected from the group consisting of calcium, magnesium, manganese, copper, zinc,sodium, potassium and lithium.

26. The method of claim 21 wherein the compound is structure 1 and the phytostanol is sitostanol.

**Figure 1**

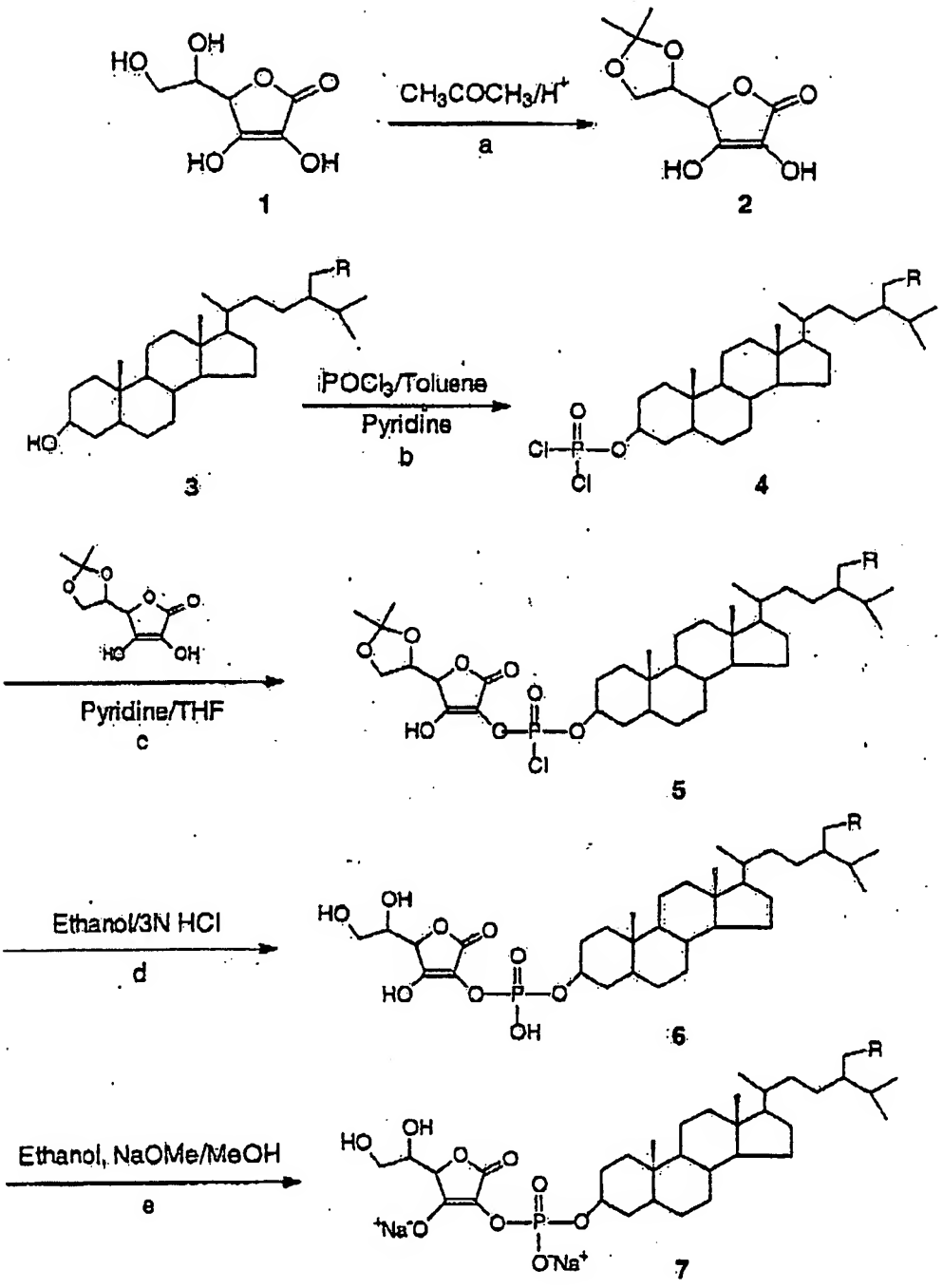

CH3COCH3/H+
a
1
2
3
POCl3/Toluene
Pyridine
b
4
Pyridine/THF
c
5
Ethanol/3N HCl
d
6
Ethanol, NaOMe/MeOH
e
7

**Figure 2**

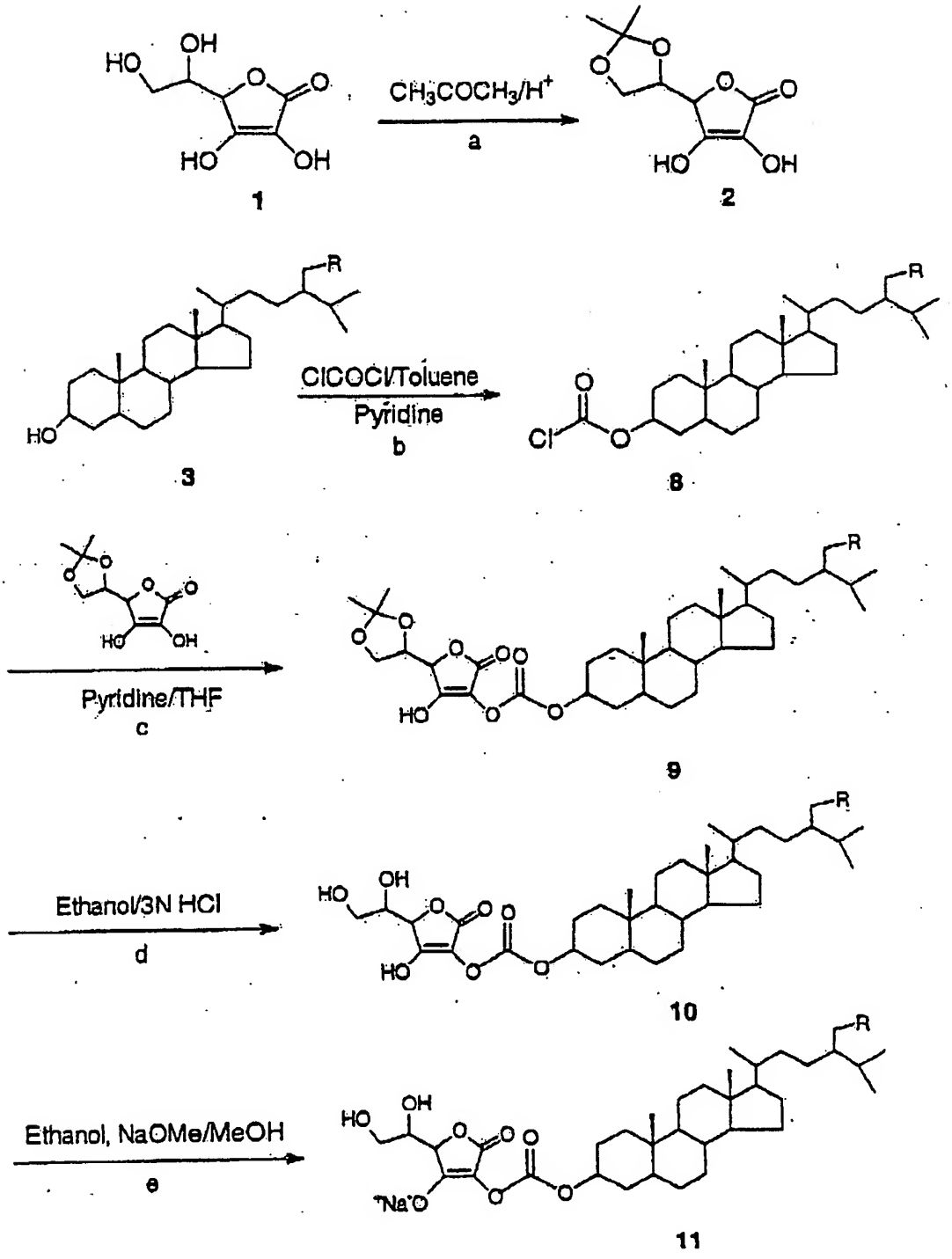

CH3COCH3/H+
a
1
2
ClCOCl/Toluene
Pyridine
b
3
8
Pyridine/THF
c
9
Ethanol/3N HCl
d
10
Ethanol, NaOMe/MeOH
e
11

Figure 3

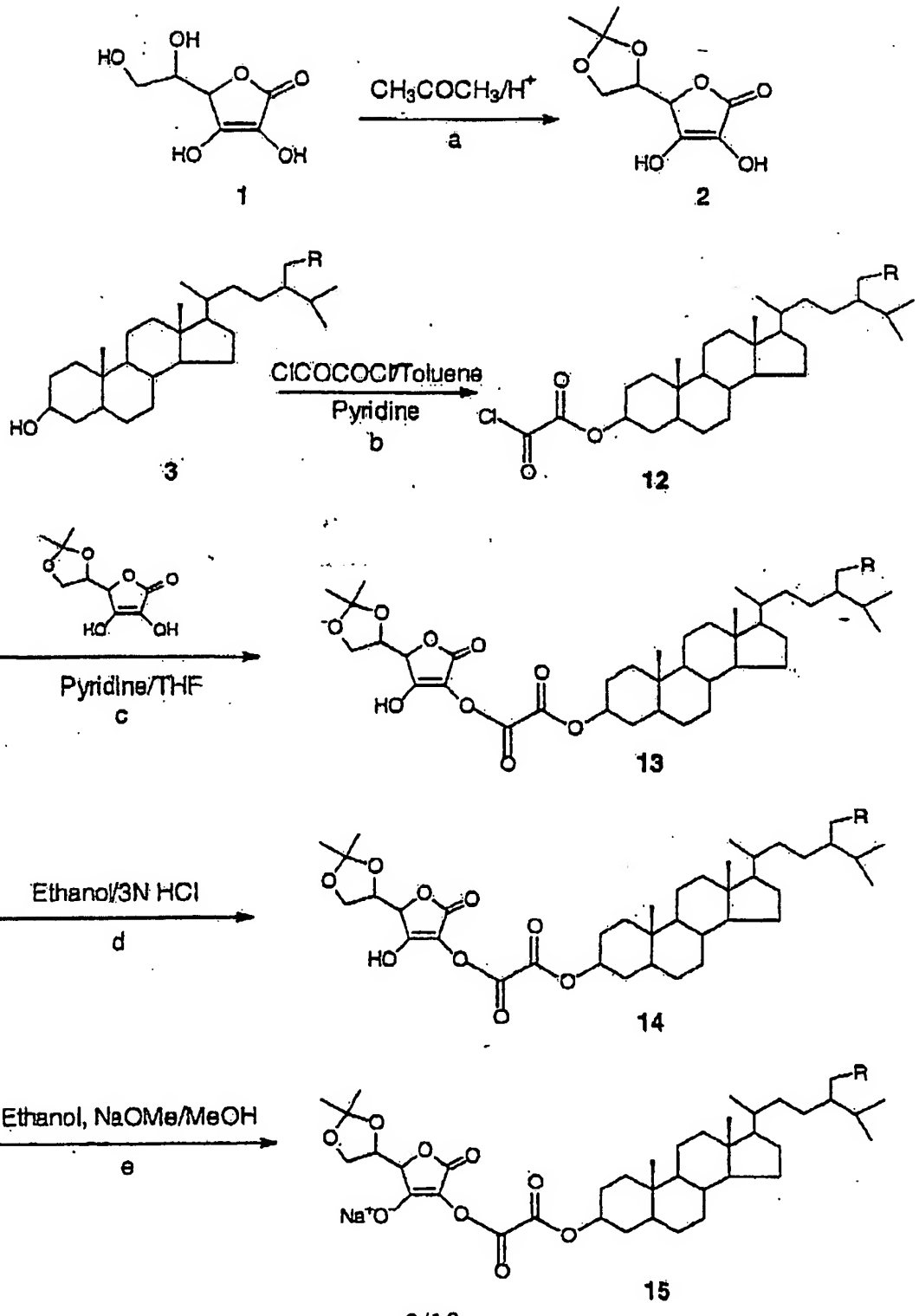

CH3COCH3/H+
a
1
2
3
ClCOCOCl/Toluene
Pyridine
b
12
Pyridine/THF
c
13
Ethanol/3N HCl
d
14
Ethanol, NaOMe/MeOH
e
15

Figure 4

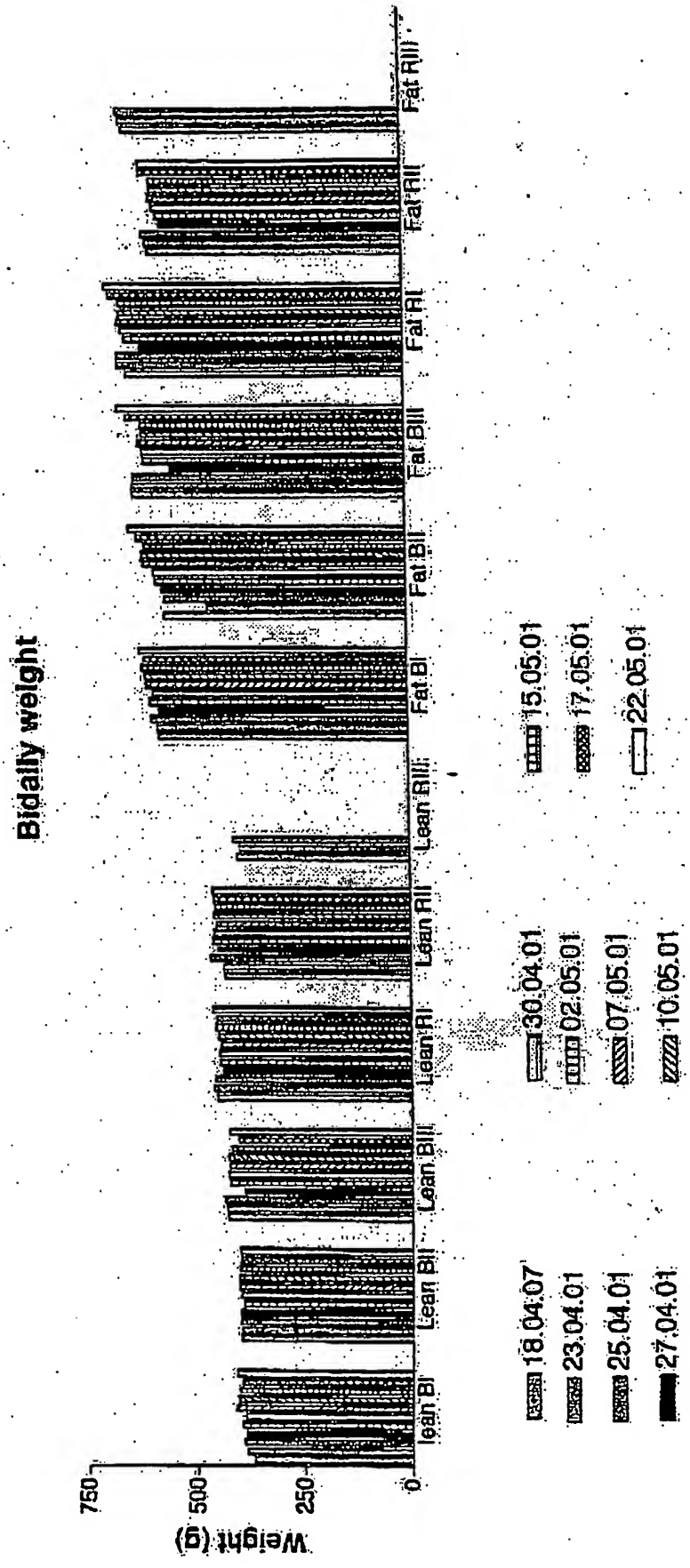

Bidaily weight
Weight (g)
750
500
250
0
Lean BI
Lean BII
Lean BIII
Lean RI
Lean RII
Lean RIII
Fat BI
Fat BII
Fat BIII
Fat RI
Fat RII
Fat RIII
18.04.01
23.04.01
25.04.01
27.04.01
30.04.01
02.05.01
07.05.01
10.05.01
15.05.01
17.05.01
22.05.01

Figure 5

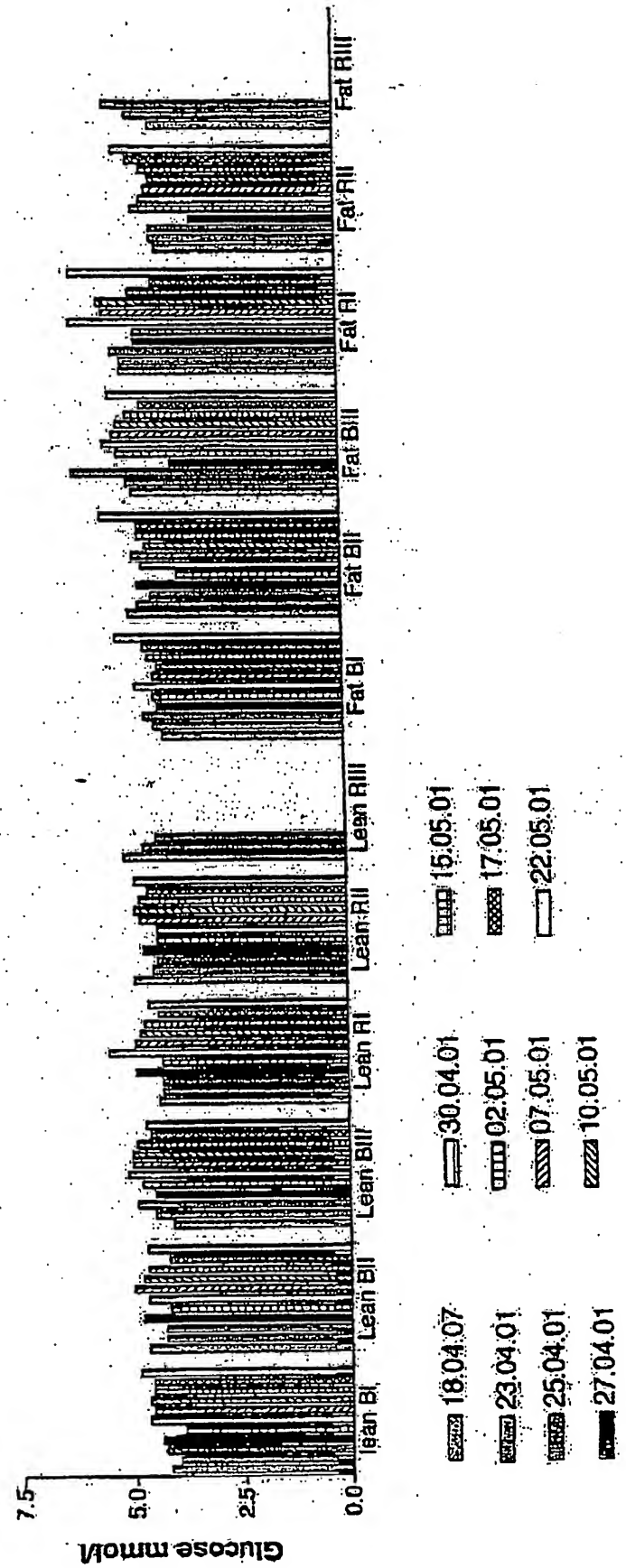

Bidaily glucose
Glucose mmol/l
7.5
5.0
2.5
0.0
Lean BI
Lean BII
Lean BIII
Lean RI
Lean RII
Lean RIII
Fat BI
Fat BII
Fat BIII
Fat RI
Fat RII
Fat RIII
18.04.01
23.04.01
25.04.01
27.04.01
30.04.01
02.05.01
07.05.01
10.05.01
15.05.01
17.05.01
22.05.01

Figure 6

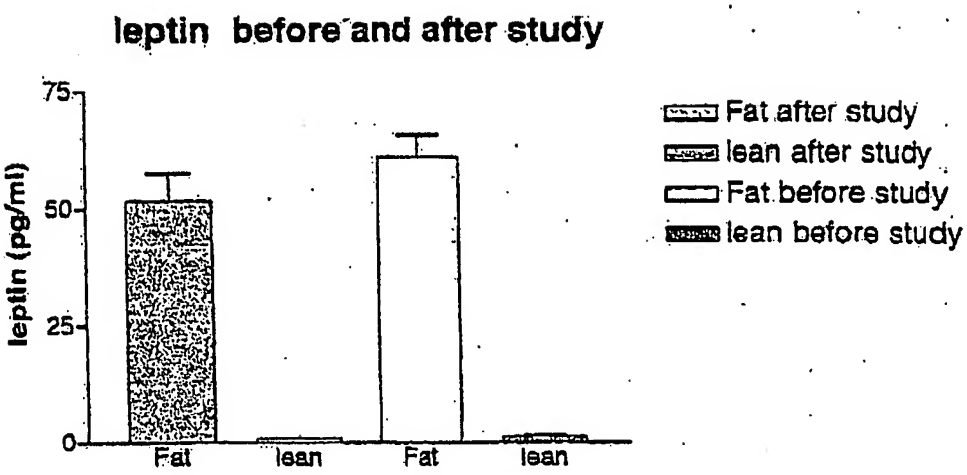

leptin before and after study
leptin (pg/ml)
75
50
25
0
Fat
lean
Fat
lean
Fat after study
lean after study
Fat before study
lean before study

Figure 7

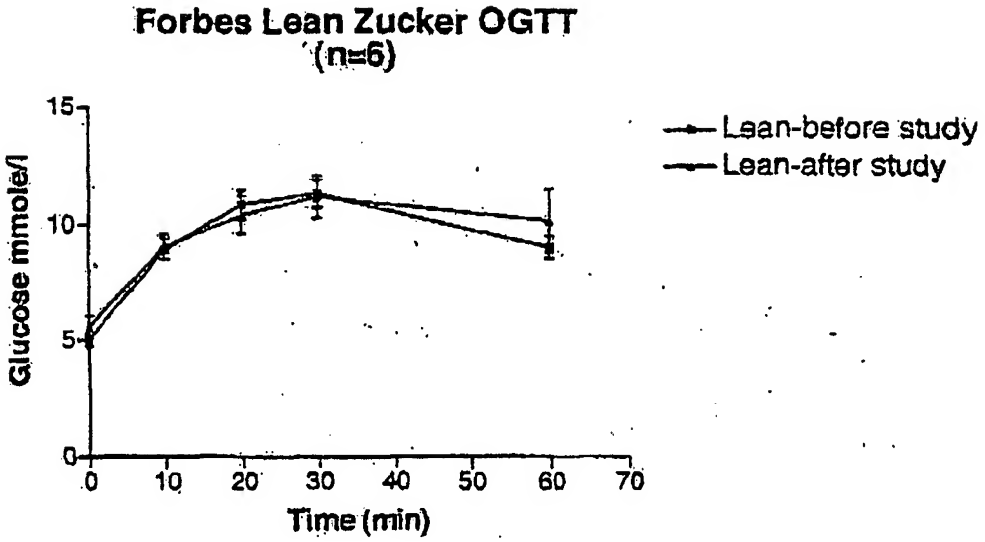

Forbes Lean Zucker OGTT
(n=6)
Glucose mmole/l
15
10
5
0
0
10
20
30
40
50
60
70
Time (min)
Lean-before study
Lean-after study

Figure 8

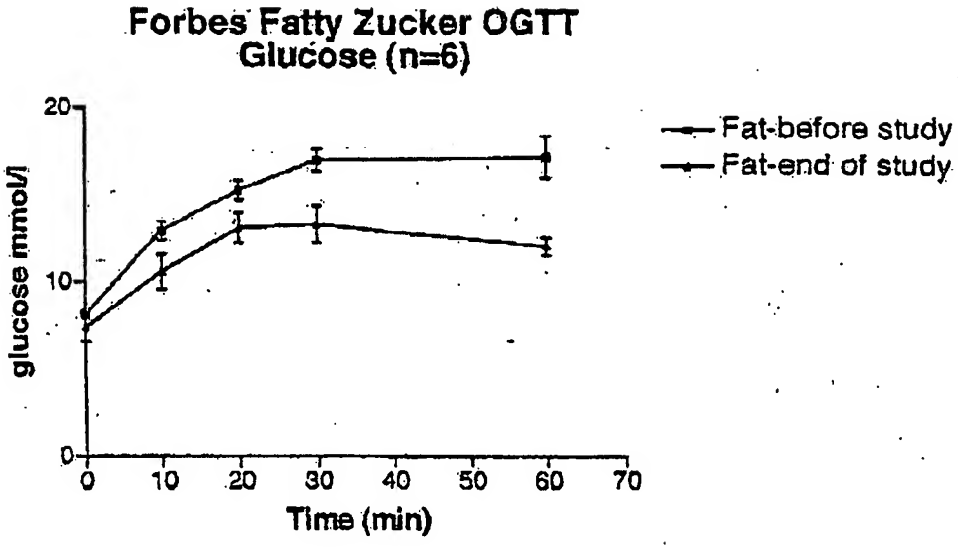

Forbes Fatty Zucker OGTT
Glucose (n=6)
glucose mmol/l
20
10
0
0
10
20
30
40
50
60
70
Time (min)
Fat-before study
Fat-end of study

Figure 9

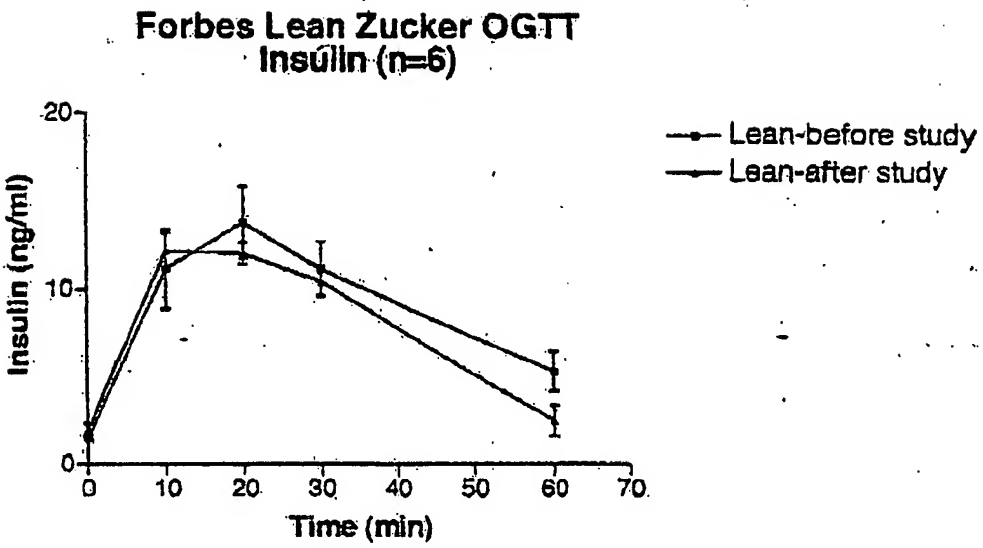
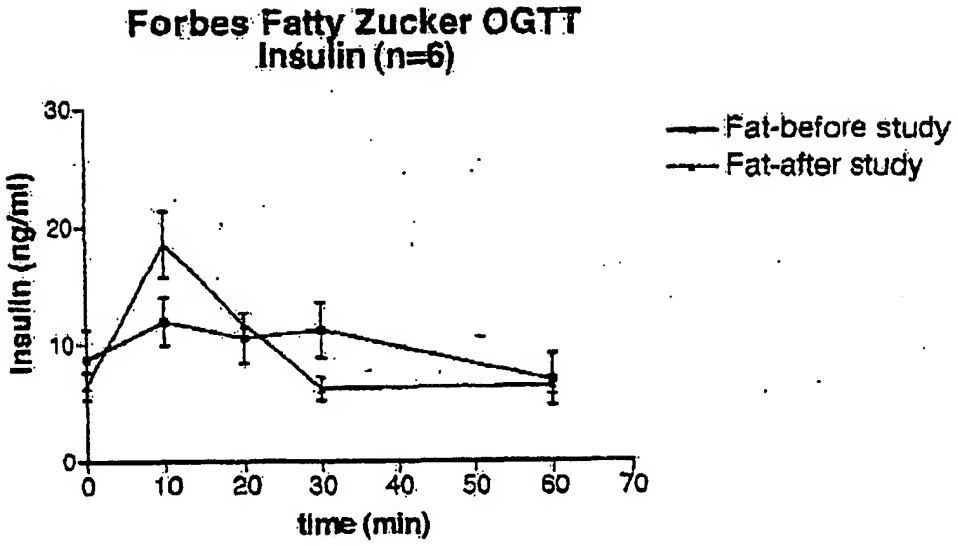

Forbes Lean Zucker OGTT
Insulin (n=6)
Insulin (ng/ml)
20
10
0
0
10
20
30
40
50
60
70
Time (min)
Lean-before study
Lean-after study

Figure 10


Forbes Fatty Zucker OGTT
Insulin (n=6)
Insulin (ng/ml)
30
20
10
0
0
10
20
30
40
50
60
70
time (min)
Fat-before study
Fat-after study

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/00369

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/575 A61K31/66 A61P5/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data, CHEM ABS Data, EMBASE, BIOSIS, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P,X | WASAN KISHOR M ET AL: "Influence of Phytostanol Phosphoryl Ascorbate (FM-VP4) on insulin resistance, hyperglycemia, plasma lipid levels, and gastrointestinal absorption of exogenous cholesterol in Zucker (fa/fa) fatty and lean rats." JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 92, no. 2, 20 February 2003 (2003-02-20), pages 281-288, XP002249505 ISSN: 0022-3549 the whole document<br>---<br>-/-- | 1-26 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 30 July 2003 | 12/08/2003 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Villa Riva, A |

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/00369

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | WO 01 00653 A (FORBES MEDI TECH INC)<br>4 January 2001 (2001-01-04)<br>abstract<br>page 3, last paragraph<br>formulae I-III<br>page 6, line 7 - line 12 | 1-26 |
| A | VANHALA M ET AL: "Relation between obesity from childhood to adulthood and the metabolic syndrome: population based study."<br>BMJ (CLINICAL RESEARCH ED.) ENGLAND 1 AUG 1998,<br>vol. 317, no. 7154,<br>1 August 1998 (1998-08-01), page 319<br>XP002249506<br>ISSN: 0959-8138<br>page 319, left-hand column, paragraph 1<br>page 319, right-hand column, line 11 - line 14 | 1-26 |

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/00369

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-2 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

**Remark on Protest**

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 03/00369

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 0100653 A | 04-01-2001 | AU 5383700 A | 31-01-2001 |
| | | WO 0100653 A1 | 04-01-2001 |
| | | CA 2377492 A1 | 04-01-2001 |
| | | CN 1370180 T | 18-09-2002 |
| | | EP 1189924 A1 | 27-03-2002 |
| | | NO 20016294 A | 22-02-2002 |
| | | US 2002156051 A1 | 24-10-2002 |